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Blood Lipid Components and SREBP-1 Gene Expression in Broiler Chickens Fed Different Dietary Lipid Sources

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ABSTRACT

***Background*:** Liver plays an important role in energy homeostasis. Intense liver diseases are accompanied with lower concentrations of n-3 and n-6 poly unsaturated fatty acids (PUFA). It has been found that n-3 PUFA play importantly protective roles in the liver. There was limited information about the effects of lipid sources on serum lipid components and liver sterol regulatory element binding-1 (SREBP-1) gene expression. Therefore, the aim of this study was to evaluate and compare the effects of fish oil, corn oil, olive oil and tallow, respectively, as dietary sources of n-3, n-6, n-9 and saturated fatty acid on serum lipid compound and liver SREBP-1 gene expression in broiler chicks.

***Materials, Methods & Results*:** In a completely randomized design, 240 one-day-old broiler chicks were allocated to five dietary treatments, four replicates and 12 chicks per each. Dietary treatments included of: control (diet without lipid supplementation) and diet supplemented with fish oil as a n-3 fatty acid source, corn oil as a n-6 fatty acid source, olive oil as a n-9 fatty acid source and tallow as a saturated fatty acid which were added to diets at 1.5, 3 and 4% in the starter, grower and finisher, respectively. At days 28 and 42 of age, liver tissue was dissected out and samples were placed in liquid nitrogen, also blood samples were collected. The SREBP-1 mRNA expression in liver tissue was quantitated using RT-PCR. Broilers fed diets containing fish oil, corn oil and olive oil as unsaturated fatty acid sources had lower concentrations of triacylglycerol than those fed other diets. Also, diets containing fish oil and tallow showed the lowest low density lipoprotein (LDL) levels at day 28 of age. Diets containing corn oil and olive oil showed higher levels of cholesterol and high density lipoprotein (HDL) than other diets but, these differences were not significant at days 28 and 42 of age ($P \geq 0.05$). In both sampling periods, the chickens fed diets containing tallow expressed higher ($P < 0.05$) SREBP-1 gene as compared to those fed other dietary lipids. Broilers fed diet containing corn oil had higher ($P < 0.05$) SREBP-1 gene expression than those fed diet containing fish oil and olive oil.

***Discussion*:** Serum lipids were affected by dietary fat source. Lower concentration of serum triacylglycerols was found in broilers fed fish oil and corn oil diets as dietary n-3 and n-6 fatty acid sources. It was shown that dietary PUFA, especially n-6 and n-3 fatty acids could reduce hepatic fatty acid and triacylglycerol synthesis. Broilers fed corn oil diet showed elevated LDL levels respect with those fed fish oil or tallow at day 28 of age. The results showed that dietary polyunsaturated fatty acids and saturated fatty acids substantially affected on SREBP-1 gene expression at days 28 and 42 of age. The birds fed fish oil as n-3 PUFA source expressed lower SREBP-1 gene compared with other groups at day 28 of age. Longer chain length, high number of double bonds, and the presence of the first double bond gives these fatty acids distinct and unique properties that separate them and their metabolic products from the more common n-6 and n-9 fatty acids. The finding of this study indicated lipogenic effects of tallow and then corn oil. The results confirmed that each of dietary lipid sources had different effect on serum lipid components. It was also found that SREBP-1 gene expression is age-dependent and it increased as age of broilers increased.

Keywords: broiler chicks, lipid type, gene expression, tallow, serum lipid components.

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INTRODUCTION

Liver plays an important role in energy homeostasis. Intense liver diseases are accompanied with lower concentrations of n-3 and n-6 poly unsaturated fatty acids (PUFA). It has been found that n-3 PUFA play importantly protective roles in the liver. Researchers have reported that dietary supplementation with fish oil (rich of n-3 PUFA) has nutritional advantages [5,32]. A complete depletion of long chain n-3 fatty acids has been observed in cardiac and liver of birds dying from sudden death syndrome [2,8]. Various studies suggest that in both birds and mammals, PUFA inhibit lipid synthesis [28,31], elevate fatty acid oxidation [26] and diet-induced thermogenesis [10] and minimize reduced growth rates by preventing the catabolic effects of immune stimulation [11]. Lipid sources added to broiler diets can modify the concentrations of triacylglycerols and lipoproteins in blood [19].

Sterol Regulatory Element Binding-1 (SREBP-1) is a transcription factor that stimulates the expression of lipogenic and cholesterol biosynthesis [34]. In rodents and pigs fed fish oil, the content of hepatic fatty acid synthase decreased by suppression of SREBP-1 expression [4, 12]. The SREBP-1 gene expression have been mainly evaluated oin mammalian species especially rodents, but researches in the birds, especially broiler chicks are rare. Therefore, this study was conducted to evaluate the effect of lipid sources containing n-3, n-6 and n-9 fatty acids on blood lipid compound and SREBP-1gene expression in broiler chicks.

MATERIALS AND METHODS

Animal and diets

A total of 240 one-day-old broiler chicks (Ross 308) was purchased from a commercial hatchery and used in a 42 days feeding trial. In a completely randomized design, chicks were divided into 5 treatments (control and four lipid sources) with 4 replicates and 12 chicks per each. Treatments included of free lipid diet as control group and 4 different levels of dietary lipids comprised of fish oil as a n-3 fatty acid source, corn oil as a n-6 fatty acid source, olive oil as a n-9 fatty acid source and tallow as a saturated fatty acid which were added to diets as 1.5, 3 and 4% in the starter, grower and finisher, respectively. Control diet had no supplemental dietary lipid and energy content was supplied by including pure starch (Tables 1 and 2). Throughout the study, chicks accessed to feed and water *ad libitum*. Lighting schedule were 23L/1D, while the temperature was gradually reduced 3˚C from initially 32˚C in each week.

Lipid analysis

Lipid from diet samples were extracted using a Folch wash of chloroform/ methanol (2:1, v/v). Methyl esters of fatty acids (FAME) in lipid extracts were prepared by transestrification using 2 mL of Heptane and 2 mL of 2M KOH-MeOH were added to the lipids, and the samples were vortex-mixed and heated at 70˚C for 10 min. The FAME were separated using a 60 m×0.25 mm i.d., 0.2 μm film thickness; TR-CN 100 column[1] and analyzed using a gas-liquid chromatograph[2] with a flame ionization detector and ultra-pure hydrogen as the carrier gas at a flow rate of 10 ml/min. Conditions for analysis of dietary lipid extracts were: spilt injection (50:1), 0.1 μL injection volume, initial oven temperature 150˚C (held for 5 min), increased to 175˚C at a rate of 5˚C/min and held for 10 min, increased to 190˚C at a rate of 10˚C/min and held for 20 min. Injector and detector temperatures were held at 250˚C and 280˚C, respectively. The peak of fatty acids were identified and quantified by comparison with the retention time and peak area of each fatty acid standard. Fatty acid content was expressed as the percentage of total fatty acids. The amounts of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), total (n-3) and (n-6) PUFA, as well as the (n-6)/(n-3) ratios were calculated from the gas-liquid chromatographic analysis.

Blood and liver tissue sampling

At days 28 and 42 of age, 8 chicks were randomly selected from each treatment (2 chicks per replicate) after an overnight fast, weighed and blood samples were obtained from wing veins and then killed by cervical dislocation. Blood samples were allowed to clot at 4˚C for 2 h and then centrifuged at 1500 × *g* for 15 min and serum was separated and stored at -20˚C until lipid compound analysis. The liver tissue was immediately dissected out and stored in a freezer -70˚C until analyzed for SREBP-1 gene expression.

Measurement of serum lipid compound

Lipid compound in serum samples were determined using triacylglycerol, cholesterol, LDL and

HDL kits[3] and an automatic biochemical analyzer[4] using colorimetric method was used 505 nm, 505 nm, 600 nm and 650 nm wave lengths for triacylglycerol, cholesterol, LDL and HDL, respectively.

RNA isolation and reverse transcription

Total RNA was extracted from the chicken liver tissue using the Trizol reagent. The concentration and quality of extraction RNA were evaluated for each sample by using both UV absorbances (260/280) in addition to 1% agarose gel electrophoresis. For estimation of RNA quantity was used nanodrop apparatus[5]. Then, RNA samples were stored at -80˚C until use. RNA was treated with DNase using Ambions DNA-free kit[6] to eliminate any possible DNA impurity. Samples were conserved at -80 ˚C until use. For optimization of Reverse and forward primers concentrations was used three different quantity for each of primers and ultimately optimized concentration was achieved for SREBP-1 and housekeeping gene primers as are shown in Table 3.

Reverse Transcription (RT) PCR was carried out using RT-PCR kit[7] containing RNA (5 µg), 1200 nM of gene specific primer and 2 µL diethylpyrocarbonate-treated water. The mixture was incubated at 65 ˚C for 5 min, then 2 µL buffer RT enzyme, 1 µl deoxy nucleoside triphosphate mix, and 400 U/µL of MMuLV Reverse Transcriptase[6] were added to the above mixture. After incubation (42˚C, 30 min), the mixture was heated (85˚C, 5 min) and then temperature was decreased to 4˚C and synthesized cDNA were quickly placed at -20˚C. Chicken SREBP-1 primer sequences were designed by oligo 7 software and 18 S rRNA primers as an internal control gene that showed in Table 4.

Table 1. Ingredients and nutrient compositions of experimental diets in grower (day 11 to day 28).

Ingredient (%)	Control	Fish oil	Corn oil	Olive oil	Tallow
Corn	51.70	55.44	55.44	55.44	55.44
Soybean Meal (46% CP)	27.40	31.70	31.70	31.70	31.70
Starch	8.70	0.00	0.00	0.00	0.74
Fish oil	0.00	3.00	0.00	0.00	0.00
Corn oil	0.00	0.00	3.00	0.00	0.00
Olive oil	0.00	0.00	0.00	3.00	0.00
Tallow	0.00	0.00	0.00	0.00	3.00
Corn Gluten	8.00	4.00	4.00	4.00	4.00
Calcium Carbonate	1.10	1.10	1.10	1.10	1.10
Di Calcium Phosphate	1.75	1.75	1.75	1.75	1.75
Sodium Chloride	0.33	0.33	0.33	0.33	0.33
DL-methionine	0.28	0.28	0.28	0.28	0.28
Vitamin and Mineral Premix*	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.25	0.25	0.25	0.25	0.25
Zeolite	0.00	1.60	1.60	1.60	0.90
Nutrient Compositions (%)					
AME (kcal/kg)	3,050	3,050	3,050	3,050	3,050
Crude protein	21.01	21.00	21.00	21.00	21.00
Crude Fat	3.91	7.18	7.18	7.18	7.18
Lysine	1.15	1.24	1.24	1.24	1.24
Methionine	0.62	0.60	0.60	0.60	0.60
Methionine plus Cystine	0.98	0.95	0.95	0.95	0.95
Fatty acids (%)					
C18:1 n-9	1.03	1.99	1.99	3.32	2.14
C18:2 n-6	2.05	2.25	3.81	2.46	2.31
C18:3 n-3	0.09	0.15	0.13	0.12	0.13
(n-6)/(n-3)	22.77	15.00	29.30	20.05	17.76

*Vitamin and mineral provided per kilogram of diet: vitamin A, 360000 IU; vitamin D3, 800000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2640 mg, vitamin B3, 4000 mg; vitamin B5, 12000 mg; vitamin B6, 1200 mg; vitamin B12, 6 mg; Choline chloride, 200000 mg, Manganese; 40000 mg; Iron, 20000 mg; Zinc, 40000 mg; Copper, 4000 mg; Iodine, 400 mg.

Table 2. Ingredients and nutrient composition of experimental diets in finisher (day 29 to day 42).

Ingredient (%)	Control	Fish oil	Corn oil	Olive oil	Tallow
Corn	56.50	59.90	59.90	59.90	59.90
Soybean Meal (46% CP)	19.00	29.20	29.20	29.20	29.20
Starch	9.86	0.00	0.00	0.00	1.00
Fish oil	0.00	4.00	0.00	0.00	0.00
Corn oil	0.00	0.00	4.00	0.00	0.00
Olive oil	0.00	0.00	0.00	4.00	0.00
Tallow	0.00	0.00	0.00	0.00	4.00
Corn Gluten	10.60	1.90	1.90	1.90	1.90
Calcium Carbonate	1.03	1.04	1.04	1.04	1.04
Di Calcium Phosphate	1.70	1.60	1.60	1.60	1.60
Sodium Chloride	0.35	0.33	0.33	0.33	0.33
DL-methionine	0.19	0.26	0.26	0.26	0.26
Vitamin and Mineral Premix*	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.41	0.15	0.15	0.15	0.15
Zeolite	0.00	1.10	1.10	1.10	0.12
Nutrient Compositions (%)					
AME (kcal/kg)	3,150	3,150	3,150	3,150	3,150
Crude protein	19.00	19.00	19.00	19.00	19.00
Crude Fat	3.94	8.30	8.30	8.30	8.30
Lysine	1.09	1.09	1.09	1.09	1.09
Methionine	0.53	0.55	0.55	0.55	0.55
Methionine plus Cystine	0.86	0.86	0.86	0.86	0.86
Fatty acids (%)					
C18:1 n-9	1.05	2.34	2.33	4.11	2.53
C18:2 n-6	2.09	2.34	4.41	2.63	2.41
C18:3 n-3	0.07	0.16	0.13	0.12	0.13
(n-6)/(n-3)	29.85	14.62	33.92	21.91	18.53

*Vitamin and mineral provided per kilogram of diet: vitamin A, 360000 IU; vitamin D3, 800000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2640 mg, vitamin B3, 4000 mg; vitamin B5, 12000 mg; vitamin B6, 1200 mg; vitamin B12, 6 mg; Choline chloride, 200000 mg, Manganese; 40000 mg; Iron, 20000 mg; Zinc, 40000 mg; Copper, 4000 mg; Iodine, 400 mg.

Table 3. Optimized primer concentrations.

	Forward Primer (nM)		
Reverse Primer (nM)	50	300	900
50	50/50	50/300	50/900
300	50/300	300/300	300/900
900	50/900	300/900	900/900

Table 4. Oligonucleotide primer sequences for RT-PCR amplification.

Primer	Sequence	Product Size (bp)
SREBP-1		
Forward	5/ - CTACCGCTCATCCATCAACG -3/	145
Reverse	5/ - CTGCTTCAGCTTCTGGTTGC -3/	
18 S rRNA		
Forward	5/ - CGCGTGCATTTATCAGACCA-3/	148
Reverse	5/ - ACCCGTGGTCACCATGGTA – 3/	

Liver mRNA quantitation by Real-Time RT-PCR

A master mix containing SYBR Green PCR master mix[8] 900 nM of forward primer, 900 nM of reverse primer, cDNA and water was prepared to perform real-time PCR. The following PCR protocol was used on the PCR ABI step-one apparatus[8]. Initial steps were 5 min at 95˚C, followed by a 3-step amplification program (95˚C at 30 s followed by 55 ˚C at 30 s and final step was 72˚C at 30 s) repeated 40 times. Quantification was performed using step-one software 2.2. The 18s RNA was considered as a house keeping. Each PCR run included a no-template control and replicates of control and unknown samples. Runs were performed in duplicate at the end of each run to control for amplification specificity; a melt curve analysis was designed.

Statistical analysis

All analyses were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS Institute. Significant differences at $P < 0.05$ statistical level compared by Duncan's multiple range tests.

RESULTS

Blood Serum Metabolites

Values for selected serum metabolites in bird fed the experimental diets are given in Table 5. Among whole of the assessed metabolites, triacylglycerol concentrations at both days 28 and 42 of age and LDL amounts at day 28 were only affect by fat sources ($P < 0.05$). Broilers fed diets containing fish oil, corn oil and olive oil as unsaturated fatty acid sources had lower concentrations of triacylglycerol than those fed other diets. Also, diets containing fish oil and tallow showed decreasing LDL levels at day 28 of age. Although, diets containing corn oil and olive oil showed higher levels of cholesterol and HDL than other diets but, this differences were not significant at days 28 and 42 of age ($P \geq 0.05$).

SREBP-1 gene expression

The effects of supplemental lipid sources on SREBP-1 gene expression in liver are reported in Table 6. Sampling results at the end of the days 28 and 42 of age showed that the birds fed tallow as saturated fatty acid source had higher SREBP-1 gene expression compared with birds fed other diets ($P < 0.05$).

Table 5. Effect of dietary various lipid sources on serum lipid compound at days 28 and 42 0f age (mg/dL).

Treatments	LDL		HDL		Cholesterol		TG	
	28 d	42 d	28 d	42 d	28 d	42 d	28 d	42 d
Control	62.4^{a}	40.0^{ab}	169	115	97.7	66.4	59.2^{ab}	36.3
Fish oil	34.8^{ab}	22.4^{b}	143	119	83.7	68.9	46.2^{b}	41.5
Corn oil	18.8^{b}	23.7^{b}	187	118	106.6	69.1	72.2^{a}	38.2
Olive oil	19.6^{b}	26.0^{ab}	171	123	100.6	75.8	54.0^{ab}	36.6
Tallow	36.0^{ab}	52.4^{a}	145	100	86.6	59.5	45.8^{b}	28.5
SEM	15.61	9.83	20.3	11.2	12.47	7.42	9.33	7.53

^{a,b}Means in the same columns with different letter are significantly different ($P \leq 0.05$).

Table 6. The relative quantification of SREBP-1 gene expression of broilers fed different fat source at days 28 and 42 of age.

Treatments	Relative Quantitation	
	28 d	42 d
Control	1.000[b]	1.000[b]
Fish oil	0.0096[d]	0.5861[c]
Corn oil	0.0198[c]	1.3266[b]
Olive oil	0.0092[d]	0.4723[c]
Tallow	2.3980[a]	24.1971[a]

[a-d]Means in the same columns with different letter are significantly different ($P \leq 0.05$).

DISCUSSION

Serum lipids were affected by dietary fat source. Lower concentration of serum triacylglycerols was found in broilers fed Fish oil and Corn oil diets as dietary n-3 and n-6 fatty acid sources. Harris [17] showed that dietary PUFA, especially n-6 and n-3 fatty acids may reduce hepatic fatty acid and triacylglycerol synthesis. In addition to, Legrand *et al*. [24] and Lochsen *et al*. [25] reported an inhibition of the activity of Δ9-desaturase, which leads to a limited triacylglycerol secretion from the liver to the blood, also, UFAs may show a higher rate of β-oxidation and as a result a higher rate of uptake of triacylglycerols from blood stream to tissue compared with SFA [31]. In recent studies, results have shown that model chylomicron-size and VLDL-size triacylglycerol-rich particles containing 5-48% of their fatty acids as EPA and DHA (derivative of α Linolenic acid) compared with n-6 triacylglycerol-rich particles were cleared faster from blood in both humans and animals [29]. Broilers fed corn oil diet showed elevated LDL levels respect with those fed fish oil or tallow at day 28 of age. Hassan *et al*. [18] reported that LDL levels was positively correlated with cholesterol and also was negatively correlated with HDL, while in our study, this relation was only shown about cholesterol amounts at day 28 of age, although these amounts was not statistically significant. Fan *et al*. [13] found that diets containing different fat sources did not affect blood cholesterol values. In general, these results agree with the findings of other authors who reported changes in the concentration of serum lipids of chickens with the dietary fat sources differing in their degree of saturation [6,30].

The current study results showed that dietary polyunsaturated fatty acids and saturated fatty acids substantially affected on SREBP-1 gene expression at days 28 and 42 of age. The birds fed fish oil as n-3 PUFA source expressed lower SREBP-1 gene compared with other groups at day 28 of age. Seo *et al*. [33] postulated that their longer chain length, high number of double bonds, and the presence of the first double bond (3 carbon atoms from the methyl terminal ,thus n-3) gives these fatty acids distinct and unique properties that separate them and their metabolic products from the more common n-6 and n-9 fatty acids. Various hypotheses about effectiveness of n-3 PUFA on gene expression through modulating cellular mechanism has been suggested by researchers. Kew *et al*. [22] reported that n-3 acyl chain position within a triacylglycerol molecule apparently has a significant effect on the potency of n-3 fatty acids in regulating protein expression. In another study, PUFA alone significantly decreased transcriptionally active concentrations of SREBP-1[35]. Worgall *et al*. [37] reported that longer chain PUFA, eg, EPA, DHA have far more inhibitory capacity on SREBP processing than do SFA have no or almost no effect on SREBP processing while results of our study was inconsistent to this finding due to broiler fed tallow as diet contained saturated fatty acids expressed much more SREBP-1 gene than other groups. Johnson *et al*. [21] with using large and small unilamellar vesicles as a model for plasma and intracellular membranes, showed that addition of fatty acids to these model membranes decreases the affinity of cholesterol for phospholipid and this causes enhanced transfer from cholesterol-rich regions to cholesterol-poor regions that would lead to ceased SREBP transport out of endoplasmic reticulum to the Golgi. The results of additional studies revealed that PUFAs can be effective on SREBP-1 gene expression by different ways: one, PUFA decreased SREBP expression by affecting the cellular composition of membranes. PUFAs accelerate the hydrolysis of plasma membrane sphingomyelin to ceramide. Sphingomyelin has a higher affinity for free cholesterol than do other phospholipids in the cell membrane. Hydrolysis of sphingomyelin to ceramide and phosphocholine impacts cellular cholesterol

homeostasis and gene transcription by 2 different mechanisms. Ceramid is a potent inhibitor of SREBP processing, a process that can regulate endoplasmic reticulum-Golgi vesicular transport and two, lower amounts of sphingomyelin result in a decreased ability to solubilize free cholesterol, which results in intracellular displacement of cholesterol and a consequent decrease in SREBP-mediated gene transcription [36].

Interestingly, in laying hens, dietary DHA as DHA oil was not effective on the expression of SREBP-1 gene in liver [7,9]. It has been speculated that in the laying hens, the *de novo* lipogenesis in the liver is genetically high in order to generate yolk [7]. The broilers that consumed control diet showed increased SREBP-1 gene expression after fish oil diet suggesting that due to this diet did not contain any supplemental oil (as unsaturated or saturated fatty acids), therefore, inhibitory effects of unsaturated fatty acid or inducible effects of saturated fatty acid did not exist and these birds expressed respect gene in a medium level. In sampling of 42 day of age, similar to previous period, tallow diet showed higher SREBP-1 gene expression with respect to other diets. But the birds fed corn oil diet as dietary n-6 PUFA source were accompanied to a progressive mechanism and thus and thus they showed elevated SREBP-1 gene expression compared with those fed other diets. In studies has been reported that mRNA expression of the key lipogenic transcription factor, SREBP-1, was inversely related to the concentration of the three potent n-3 PUFA, ALA, EPA and DPA and positively related to n-6 PUFA concentration [1,3].

The findings of a study [27] also suggested that the anti-lipogenic effects of n-3 PUFAs are less potent than the prolipogenic actions of n-6 fatty acids. These results are supported by researcher that showed higher intake of LA was associated with reduced conversion of ALA to downstream long-chain derivatives [20,23]. With regarding these results are inconsistent with our finding, we can possibly conclude that due to in the end of experimental period (42 day of age), concentration of dietary n-6 PUFA in corn oil was maximum, also it is preventive effects on n-3 PUFAs was more severe and thus derivatives of n-3 PUFAs were less produced and their suppressive effect on SREBP-1 gene expression was decreased.

CONCLUSION

Dietary various lipid sources had profound effects on serum TG and LDL of broilers. In addition, the birds fed diet containing tallow expressed higher SREBP-1 gene than those fed dietary unsaturated sources.

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Ethical approval. Use of birds in this study was approved by the local institutional animal ethical committee (Protocol 17-16-5-10938; 90-11-15).

Declaration of interest. The authors report no conflict of interests. The authors alone are responsible for the content and writing of the paper.

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